12-11-2013

The Effect of Varying Frequencies of Mechanical Vibration on the Rate of Orthodontic Tooth Movement in Mice

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A Thesis

Submitted in Partial Fulfillment of the

Requirements for the Degree of

Master of Dental Science

At the

University of Connecticut

2013

APPROVAL PAGE

Masters of Dental Science Thesis

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2013

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ABSTRACT

Objective: The aim of this study is to utilize the Orthodontic Tooth Movement (OTM) model in a mouse in order to study the mechanical vibrational effects in bone. Specifically, we wish to test various frequencies of vibration under an orthodontic force to determine whether or not an increase in OTM is seen. We also intend to further investigate the role of osteoclasts in OTM and how they interplay with increasing tooth movement.

Materials and Methods: Fifty-eight male CD1 mice were randomly placed into 1 of 8 groups. Three of these groups were part of the experimental subset which all received an orthodontic force in conjunction with either 5Hz, 10Hz or 20Hz vibration. The 5 control groups consisted of matching vibrations groups without the presence of an orthodontic force, along with a baseline control group and an orthodontic force only group. The orthodontic force application consists of a 10g Ni-Ti closed-coil spring connecting the maxillary right first molar and the maxillary central incisors, which is kept in place with steel ligatures at either end for a total of 14 days. During this time period, any mice that were part of a group that required mechanical vibration were then exposed to a vibratory force from a Bose Transducer to the occlusal surface of the molar every 3 days for 15-minute sessions. All animals were then sacrificed and underwent micro-CT analysis followed by histological staining for identification of osteoclasts in the area surrounding the maxillary first molar.

Results: After 14 days of orthodontic force application, there was no difference in tooth movement between the different experimental groups. However, the maximum tooth movement was observed in the spring +5Hz group and was least in the spring only group. Micro-CT analysis showed a statistically significant decrease in bone volume fraction (BV/TV) when control groups were compared with experimental groups; however, differences in bone volume and tissue density were statistically insignificant between different experimental groups.

Conclusion: From the findings of this study, we can conclude that mechanical vibration has no statistically significant effects on the amount of orthodontic tooth movement seen in a mouse model. However, we are currently increasing the sample size in hopes that a certain frequency will show a preferential finding**.**

BACKGROUND

Anatomy, Biological Responses, and Orthodontic Tooth Movement

Orthodontic treatment in recent years generally approximates about two years of fixed appliance therapy in order to complete treatment. During this time all biological processes in close proximity can be affected in some fashion, with detrimental effects in some cases. While in treatment, patients have an increased susceptibility to periodontal disease, dental caries and root desorption, all of which become more severe if treatment time is prolonged. As a consequence, anything that can help reduce orthodontic treatment time is both beneficial for patient and practitioner. Due to the fact that Orthodontic Tooth Movement (OTM) is the result of gradual remodeling (cycle of apposition and resorption) of supporting alveolar bone, factors affecting this cycle could modulate the rate of tooth movement [1].

The attempt to shorten the patient's treatment time can be divided into 2 main categories: pharmacological and mechanical. Local or systemic administration of biological factors [2, 3] such as parathyroid hormone (PTH) [4], thyroxin [5], Vitamin D3, [1,25 $(OH)_2D_3$] [6] and prostaglandins [7] have been investigated in prior experiments. The problem with such a systemic approach to accelerating tooth movement, however, lies in the numerous adverse reactions, such as, local pain [8], severe root resorption [9], and other drug-induced side effects. For this reason, the trend has turned towards finding a physical or mechanical approach in the hopes that side effects can be avoided. These approaches include, but are not limited to: electrical currents [10, 11], magnets [12], laser beams [13] and various types of vibration at different frequencies [14-16].

With respect to vibration, however, the literature is limited, contradictory, and there are many areas that are still lacking sufficient information. At the current time there have been experiments in various animal models and even in humans, but there is still a disparity in the effects seen, the type of vibratory stimuli utilized and no one has elucidated the ideal frequency for optimal response, if such a frequency even exists. The types of vibration that have been looked at thus far include: whole-body vibration, pulsed electromagnetic field driven vibration, resonance vibration, and mechanical vibration.

Studies that involve whole-body vibration have been done in both animals and humans. Christiansen and Silva studied the effect of this type of vibratory stimuli on forty adult mice using a frequency of 45 Hz with varying magnitudes of force for fifteen minutes per day for a total of 5 weeks. They were able to find an increase in Orbicular bone volume in the experimental vibration group, however it was not dose-dependent [17]. Rubin et al. turned their investigation over to humans where they carried out a 1-year prospective, randomized, double-blinded, placebo-controlled clinical trial on seventy post-menopausal women. In these subjects they administered whole-body vibration at a frequency of 30 Hz with 0.2 grams of magnitude for twenty minutes per day. They found an inhibition of bone loss in both the spine and the femur with more significant findings in subjects whose body mass was lower [18]. It appears from these studies that low-magnitude, high frequency vibration for relatively short durations has an anabolic potential for bone, namely, it increased the number and width of trabeculae as well as enhancing the stiffness and strength of cancellous bone [18]. Due to the fact that the same molecular mechanisms involving bone turnover, specifically modeling and remodeling are similar to those that are required for OTM, it makes sense that applying a vibratory stimuli might have an effect on the rate of tooth movement.

Various studies have looked at applying a pulsed electromagnetic field (PEMF) in order to create a vibratory stimulus. As far back as 1987, Stark and Sinclair looked at applying PEMF in forty male Hartley guinea pigs, where they applied 12 grams of orthodontic force and looked at the effects of a 25 Hz PEMF for ten days. They found a significantly increased rate and total amount of tooth movement along with a significant increase in bone and matrix deposition and in the number of osteoclasts present [16]. Darendeliler, Sinclair and Kusy in 1995 also studied PEMF but incorporated a samarium-cobalt magnet as well. In their study they looked at a frequency of 15 Hz while applying an orthodontic force of 15 grams. At the end of the 10-day experiment, they found that the amount of tooth movement in the magnet and PEMF groups was significantly greater than that of the group with orthodontic force alone [12]. They hypothesized that the increase in the rate of OTM was due to a reduction of the initial lag phase which follows force application [12]. Again in 2007, Darendeliler et al. looked at the effects of PEMF and neodymium-iron-born magnets using forty-four Wistar rats. This time they applied 25 grams of orthodontic force with a frequency of 30 Hz. Their results showed a significantly greater tooth movement in the group exposed to the PEMF [14].

Nishimura et al. looked at the effects on orthodontic tooth movement in rats utilizing resonance vibration (vibration with a continuously changing frequency) applied to the dentition[15]. In their 21-day study they used forty-two male Wistar rats, which were divided into two groups. A 0.012 Nickel-Titanium (Ni-Ti) expansion spring provided an orthodontic force of 12.8 grams, while resonance vibration (60 ± 8 Hz) was applied to the occlusal surface of 1^{st} molars for 8 minutes, one time per week. At the completion of the experiment, they found that the amount of tooth movement in the vibration group was significantly greater (15%). Histologically, they found that on day 3 there was enhanced Receptor Activator of Nuclear factor kappa-B ligand (RANKL) expression by osteoclasts and fibroblasts, and a significantly increased number of osteoclasts present (1.7x control) on day 8 [15].

Ultrasonic vibration has also been studied with its effects on OTM. Ohmae et al. looked at 5 adult male beagle dogs where they bilaterally extracted maxillary first premolars. They then applied an 80-gram force using a sectional archwire between the canine and first premolar in order to close the extraction space. During this time, one side was exposed to a homo-directional ultrasonic vibration for 2 minutes, two times per week for a total of 8 – 10 weeks. They too found a significantly greater amount of tooth movement in the teeth exposed to ultrasonic vibration [19].

During the last few years a company by the name of AcceleDent™ has produced a device that can be used in humans in order to apply a vibratory force of 30 Hz to the dentition. To date they have conducted two studies. The first was a non-controlled experiment in 14 subjects where they used the appliance for 20 minutes per day for a total of 6 months. While they had no control to compare their results to, they postulated that the 3mm per month that they saw in the maxilla and the 2.1mm per month in the mandible is greater when compared with the accepted norm of approximately 1mm per month often seen clinically [20]. Following these findings they then conducted a prospective, randomized, blinded, sham-controlled clinical trial on 39 subjects at the University of Texas at San Antonio, which found promising results, but is yet to be published in the orthodontic literature. They found significantly greater tooth movement during the aligning phase (106%) and significantly greater tooth movement during space closure (38%).

Since the release of this commercially publicized vibratory apparatus there has been an increase in interest in the effects of vibration on OTM. Another company has produced a similar product, named the "Tooth Masseuse", however it's price point is considerably cheaper, on the order of 25 fold less. This company has mainly advocated its use for reducing the pain associated with orthodontic tooth movement, but recently a prospective randomized clinical trial by Miles et al. was performed in order to assess the Tooth Masseuse's ability to increase the rate of OTM as well as alleviate the patient's discomfort. As with all studies, again a different frequency and force magnitude is produced by this machine, namely 111 Hz and 6 grams for 20 minutes per day. They, however, found that the appliance was unsuccessful in having any effect on either aspect and concluded that at least at this frequency, the application of mechanical vibration has no clinical advantage [21].

Recently at the University of Connecticut Health Center a project was undertaken where the effects of mechanical vibration on OTM was studied in 37 female Sprague Dawley rats. In this experiment an orthodontic force of 25 grams and two different frequencies were applied: 30 Hz and 60 Hz. The vibratory force was applied for 10 minutes two-times per week for a total duration of 14 days. The results of this study, however, are very different from that seen in prior experiments. Rather than an increase in the amount of OTM, they saw a significantly reduced amount of tooth movement (50%) in the 30 Hz group, along with a significantly greater number of apoptotic cells. These findings pose a very different outlook on the effect of vibration on OTM, and thus further investigation is clearly needed. The results of these findings were utilized as a pilot study for a continuation of another experiment by the same researchers where they looked specifically at 30 Hz only with a force of 0.4 grams and keeping all other parameters the same in 26 female Sprague-Dawley rats. Their findings were again inhibitory in nature, showing a significant reduction in the amount of orthodontic tooth movement when 30 Hz vibration was applied [22].

It is clear from all of these studies, that the application of a vibratory stimulus does have an effect on the metabolism of bone, and thus could play a role in the rate of OTM. However, there is still much to be learned in this field, and further research is clearly needed in order to further understand this phenomenon. The purpose of this study is to assess the effects of varying frequencies of mechanical vibration on the rate of orthodontic tooth movement in a mouse model.

Tooth Movement Models

Historically, several animal models have been designed to study tissue responses to mechanical loading during orthodontic tooth movement. Primate, dog and cat models have been reported in pioneering histological studies using light microscopy [23, 24] and electron microscopy [23, 25]. The limitations related to the use of these animal models are directly due to their similarity and applicative value to humans. The rat model proposed by Waldo in 1954 [26] had increased levels of experimental control over other animal models and has become the investigative workhorse for unraveling the processes of mechanotransduction and alveolar bone remodeling in orthodontic tooth movement [27]. Today, rats are the most commonly used animal models, accounting for over half of all orthodontic tooth movement animal studies [27]. Compared with most other animals, the use of the rat has several advantages: they are relatively inexpensive, which allows using large samples; they can be housed for long periods of time; histological preparation of the rat is easier than other models; there is greater availability of antibodies required for cellular and molecular biological techniques, and they are larger than mice, which makes it easier to place orthodontic appliances. The rat does have its own limitations however: denser alveolar bone as compared to humans; lack of osteons and less abundant osteoid tissue; structural dissimilarities in the arrangement of PDL fibers and the supporting structures, and tissue development during root formation and tissue changes as a result of orthodontic treatment appear to be faster in rats than in humans, although their principal mechanisms are the same [27].

Rat models have enabled a diverse scope of orthodontic research, ranging from measuring proliferation rates of periodontal cells under load, to assessing the effects of prostaglandins, bisphosphonates and leukotrienes on tooth movement [7, 28, 29].

In Ren et al.'s systematic review of the 153 (57% of the total tooth movement models) studies done on rats over the past twenty years, however, it was found that the majority of the experimental models utilized poorly designed force systems that lacked control over force level consistency over the duration of tooth movement [27].

Only three methods met Ren's inclusion criteria for a good model [27]. Ren's inclusion criteria were: a force magnitude of less than 20cN; mesial movement of molars; an experimental duration greater than 2 weeks; and no extra experimental conditions, such as drug intervention. Most of the studies failed to take into account the physiology of the rat (i.e. natural distal drift of the molars and the continual eruption of the incisors), or the orthodontic appliance design was faulty. The distal drift of the molars underestimates the amount of mesial movement of the molars and the continual eruption of the incisors can lead to a minimized control of force direction. The appliance design can be considered poor when it does not take into account the 50-fold reduction in the rat's molar root surface area compared to humans, or the appliance simply lacks a constant and continual force [27].

Pavlin et al. were the first to develop a mouse model back in 2000, where they performed experiments to test the load conditions that would generate an optimal biological response of paradental tissues [30, 31]. They used an elastomeric "o-ring" tied between maxillary incisors and the first molar, and a red elgiloy (alloy of nickel and cobalt) open coil spring (0.0056" x 0.022", Rocky Mountain Orthodontics, Denver, CO) tied and bonded to the same teeth, respectively. It was found that the coil spring had considerable advantages over the "o-ring." Firstly, bonding of a coil spring to the molar and the incisors eliminates contact of the appliance with gingival tissues, greatly reducing the risk of tissue irritation [30, 31]. This correlates with the criticisms of Charles Waldo, whom in 1954, was among the first pioneers responsible for the advent of the rat model. His method, known as the Waldo method, utilized an orthodontic intermaxillary elastic, which was stretched and inserted into the interproximal space just cervical to the contact area between the molars of rats [26]. This method has been criticized due to the unknown force decay of the elastic. Springs have proven to be more reliable because they are able to deliver a reproducible force of 10 +-2cN over a range of 3-15mm of activation [27]. Secondly, the spring has a lower force/deflection rate (F/Δ) as compared to an elastomeric. These two major factors allow for a more precise and reproducible application of a low level force, which also remains more constant compared with that delivered by an elastomeric "o-ring."

King [32], Keeling [33], and Nixon [34] in the 1990's produced the only 3 articles that met all of Ren's criteria for an ideal rat model [27]. Forces of 20, 40, and 60cN were used in all 3 articles. These studies were criticized for having an initial constant force, but not reactivating it, and forces of 40 and 60cN being too high. The appliance consisted of a 9 mm length of closed coil spring (0.006" Hi-T; arbor diameter: 0.022", Unitek, Monrovia, Calif.) suspended between a cleat bonded to the occlusal surface of the maxillary first molars and the lateral surface of the maxillary incisors. Initial force values were measured by suspending known weights from the anterior end of these coils before fixation to the incisors. Tooth movement was based from enlarged cephalograms, and was measured from the position of a reproducible landmark on the molar cleat with respect to either zygomatic amalgam implants, or a barbed broach placed submucosally on the palate. Palatally placed barbed broaches represented a more reliable, less traumatic, and more easily executed superpositional landmark than zygomatic amalgams which had a 79% appliance success rate with many animals ending up losing too much weight. All of these factors contributed to poor overall animal care [27, 32-34].

In 2004, Ren's model was fabricated due to the shortcomings of the rat models used from 1981-2002, and was used as a spilt-mouth design. This design compensated for the physiological distal drift of the molars, growth of the snout and concomitant forward movement of the incisors, and the continuous eruption and possible distal tipping of the incisors. In this model stainless steel ligature wires with a diameter of 0.2 mm were bent to enclose all three maxillary molars as one unit. To this ligature wire a Sentalloy®

closed coil spring (Ni Ti, 10 cN, wire diameter 0.22 mm, eyelet diameter 0.56 mm, GAC, New York, USA) was attached to deliver a reproducible force of 10 ± 2 cN over a range of 3-15 mm of activation. A transverse hole was drilled through the alveolar bone and both maxillary incisors at the mid-root level using a drilling bur (D0205, Dentsply). A stainless steel ligature wire (diameter 0.3 mm, Dentaurum) was inserted through the hole. Bonding was applied until the buccal and palatal wires were entirely embedded in the bonding material, after which it was light cured. It was activated and subsequently attached to the ligature wire through the snout and the incisors [27].

Most recently, in 2006, Yoshimatsu et al used a variation of the Ren model, but instead used Ni-Ti coil springs [35] in order to further develop the mouse model for OTM. Their mouse model included a Ni-Ti closed coil spring, with the wire diameter of 0.15mm, and a coil diameter 0.9mm. The appliance was inserted between the maxillary incisor and the first molar on the left side. It was fixed with a 0.1mm wire around each tooth using a dental adhesive agent (Superbond; Sunmedical Shiga, Japan). To prevent detachment from the maxillary incisors during the experiment, a shallow groove, 0.5mm from the gingiva, was made on the maxillary incisor every 4 days, and the wire was reattached at the new groove. According to the manufacturer's database, the force level of the coil spring after activation was approximately 10g. The maxillary left molar was used as the experimental side, and the right as the control, taking into account the distal molar drift that would naturally occur [35].

RATIONALE

It has been well documented that high-frequency, low-magnitude vibration has an anabolic effect on bone, namely an increase in trabecular bone, but much is unknown about the specific mechanisms involved during this modeling and re-modeling process. Previous studies in animal models have shown that vibration can increase OTM when coupled with an orthodontic force, but an optimal frequency and an optimal force of the vibration has not been established. Most vibrational studies regarding OTM have been conducted in guinea pigs and rats, which are genetically similar to humans but not as close as mice. Therefore the purpose behind this study is to utilize the OTM model in a mouse in order to study vibrational effects in bone. Specifically we wish to test various frequencies of vibration under an orthodontic force that is equivalent to the force used clinically in humans to determine whether or not an increase in OTM is seen. We also intend to further investigate the role of osteoclasts in OTM and how they interplay with increasing tooth movement

HYPOTHESIS

We hypothesize that mechanical vibration will increase the rate of OTM when applied directly to the dentition. As a result, greater amounts of tooth movement will be seen when the teeth are directly measured utilizing μ-CT images taken from the mice, following their euthanization. We also hypothesize that an increase in vibrational frequency will cause an increase in osteoclast number based on a dose-response, which will be evaluated by utilizing specific immunohistological analyses.

Null Hypothesis 1: There will be no difference in the amount of OTM in the experimental vibration groups versus the force-only control.

SPECIFIC AIMS

Specific Aim 1: To utilize the current *in vivo* mouse model for OTM to measure the difference in the amount of tooth movement when varying the frequency of vibration under a constant force.

Specific Aim 2: To determine the optimal frequency of vibration during OTM for maximal osteoclast recruitment and proliferation.

Specific Aim 3: To quantify and compare the bone volume, tissue density and bone volume fraction between different control and experimental groups.

MATERIALS AND METHODS

Study Design

All experimental procedures were performed at the University of Connecticut Health Center under the strict guidelines of an approved protocol (ACC# 100340-0115) for animal experimentation. The study consisted of 58 male CD1 mice (12 weeks old), which were randomly placed into 1 of 8 groups (3 experimental / 5 control). In each group the procedure will be applied to the right side of the maxilla.

The following are the 3 experimental groups:

(1) Spring + 5hz Vibration (n = 10)

(2) Spring + 10hz Vibration (n = 10)

(3) Spring + 20hz Vibration (n = 10)

The following are the 5 control groups:

(1) No Spring + No Vibration (n = 5)

(2) No Spring + 5hz Vibration Only (n = 5)

(3) No Spring + 10hz Vibration Only (n = 5)

(4) No Spring + 20hz Vibration Only (n = 5)

(5) Spring Only (n = 8)

Method for Orthodontic Force Application

Animals were anesthetized with an intraperitoneal injection of ketamine and xylazine (6μL/g body-weight). A custom mouth-prop was fabricated from 0.036 mm SS wire and was placed between the maxillary and mandibular incisors in order to hold the mouth open.

Mice that were subjected to an orthodontic force had a Nickel-Titanium (Ni-Ti) coil-spring placed between the central incisors and the maxillary right first molar. Specifically, a low force/deflection rate Ni-Ti closed coil-spring (G&H wires, Indianapolis, IN) was placed and activated 1.5mm delivering a continual force of approximately 10g. The force/deflection rate (F/Δ) for the spring was determined in order to calibrate the amount of force produced by activation of the spring.

Prior to appliance delivery, Ni-Ti coil spring appliances were pre-fabricated consisting of two separate segments of 0.004 mm stainless-steel (SS) ligature wire, one connected to either end of the Ni-Ti coil spring (wrapped around two coils).

In order to connect the spring appliances, one end of the 0.004mm SS ligature wire was threaded through the contact between the first and second right maxillary molars, and then cinched tightly around the molar below its height of counter. The spring was then activated to the incisors where the other 0.004mm SS ligature wire was cinched tightly around both maxillary central incisors. To prevent any dislodging, the wire around the incisors was secured using composite resin (Transbond XT Light Cure Adhesive Paste, 3M Unitek, Monrovia, CA), which was cured using a commercial unit (LEDemetron 1, Dentsply). Finally, the mandibular incisors were reduced slightly in length to try and reduce the amount of appliance breakage when the mice were eating [35].

After appliance insertion the mice were allowed to recover in the presence of an incandescent light for warmth and the animals were then returned to their cages once full ambulation and self-cleansing had returned. The appliance was checked every 3 days, and additional bonding material was added if necessary. The duration of the experiment was 14 days.

<u>Application of Mechanical Vibration</u>

Following adequate induction of general anesthesia using a mixture of ketamine and xylazine (described above), a custom mouth-prop fabricated from 0.017” x 0.025” Titanium Molybdenum Alloy (TMA) wire was placed between the maxillary and mandibular incisors in order to hold the mouth open. At this point, a feedback-loop controlled, electromechanical actuator (Model 3230, Bose/EnduraTec, Minnetonka, MN) was utilized in order to apply unilateral mechanical vibration to the occlusal surface of the maxillary right first molar along the long axis of the tooth. Loading protocols for individual animals consisted of 15 minutes of mechanical vibration, at 5, 10 or 20 Hertz (cycles/second) depending on the group the mouse was assigned to. Mechanical vibration was applied at three-day intervals (day: 1, 4, 7, 10, 13).

Wellness Monitoring and Euthanasia

Depending on the group the mice were randomly assigned to, they were exposed to orthodontic force, mechanical vibration or the combination of the two, or no treatment at all. Prior to any experimentation, all mice were acclimated to a 12-hour light/dark cycle for at least 1 week.

All animals were housed under normal laboratory conditions and were a fed soft dough diet (Bio-Serve Frenchtown, NJ) and water ad libitum. In order to monitor the food intake during the experiment, all mice were weighed every 3 days. Any mouse that lost more than 20% body-weight was sacrificed and excluded from the study.

Upon completion of the experiment (day 14), all mice were euthanized by CO_2 inhalation. All animal experimental procedures were in compliance with the guidelines set forth in the Guide for Care and Use of Laboratory Animals [36].

Micro-CT Analysis and Tooth Movement Measurements

Following euthanasia, at day 14, the mice were decapitated and cleansed of soft tissues. The skulls were then placed in 10% neutral buffered Formalin for seven days at +4°C with constant agitation, upon which time they were sent for radiographic imaging. Specifically, three-dimensional images were obtained using a micro-focus X-ray computed tomography (micro-CT) machine. All micro-CT imaging and subsequent analysis was performed by the Micro-CT facility, located in The Medical Arts and Research Building (MARB) at the University of Connecticut Health Center.

Scanning was performed at 55 kV and 145 amps, collecting 1,000 projections per rotation at 300 millisecond integration times. Three-dimensional images were then constructed using standard convolution and back projection algorithms with Shepp and Logan filtering and rendered within a 12.3 mm field of view at a discrete density of 578,704 voxels/mm 3 (isometric 12 mm voxels).

The images obtained were then utilized to determine the amount of orthodontic tooth movement by measuring the distance between the maxillary first and second molars. The two points that were used were the most distal point of the first molar (M1) and the most mesial point of the second molar (M2), with the difference (M1-M2 distance) being the total distance the tooth moved. These measurements were made in the sagittal plane along the path of the tooth movement, which was located by determining which image plane showed the most root structure.

The initial separation distance (day 0) was 0 mm in all groups, which means that the most convex surfaces of both molars were in contact with each other prior to the application of any orthodontic force.

The region of interest for the analysis of bone volume, tissue density and bone volume fraction (BV/TV) consisted of a square region that extended 200 µm from the mesial surface of the disto-buccal and disto-lingual roots of the right maxillary first molars.

STATISTICAL ANALYSIS

Simple descriptive statistics were used to summarize the data. Outcomes examined in the experimental groups included inter-molar distance, bone volume, tissue density and bone volume fraction (BV/TV), whereas the outcome examined in the control groups: Control 1: (No Spring + No Vibration); Control 2: (No Spring + 5hz Vibration); Control 3: (No Spring + 10hz); Control 4: (No Spring + 20hz vibration) included bone volume, tissue density and Bone Volume Fraction (BV/TV).

Considering the small sample size, non-parametric tests were used to examine the association between the outcome variables and treatment groups: Spring Only (control); Spring + 5hz; Spring + 10hz; and Spring + 20hz. Kolmogorov-Smirnov test was used to examine the distribution of outcome variables and the distribution was assessed both in the control and treatment groups. Kruskal Wallis tests were used to compare the outcomes across treatment groups. Pairwise comparisons between different groups were conducted using the Mann-Whitney U test.

All statistical tests were two sided and a p-value of <0.05 was deemed to be statistically significant for the Kruskal Wallis test. Considering the multiple pairwise comparisons used, in-order to minimize Type 1 errors, Bonferroni corrections were used.

RESULTS

All the mice, except five were included in the study. All five of the excluded mice were removed primarily due to the loss of the orthodontic appliance. All mice included in the study remained healthy and had a slight increase in body weight.

Tooth movement

After 14 days of orthodontic force application, there was no difference (statistically insignificant) in tooth movement between different experimental groups. However, the maximum tooth movement was observed in Spring + 5Hz group and was least in Spring Only group (Spring + 5Hz > Spring + 10Hz > Spring + 20Hz > Spring Only).

Micro-CT analysis showed a statistically significant decrease in bone volume fraction (BV/TV) when control groups (No Spring + No Vibration [base line], No Spring + 5Hz, No Spring + 10Hz and No Spring + 20Hz) were compared with experimental groups (Spring + 5Hz, Spring + 10Hz, Spring + 20Hz and Spring Only), however, between the four different control groups and four different experimental groups there was no statistical difference in bone volume fraction. Among the control groups, the bone volume of baseline control was significantly greater ($P<0.05$) than No spring + 20 Hz group, however, the tissue density was not different ($P>0.05$) between the control groups. Similarly, the differences in bone volume and tissue density were statistically insignificant between different experimental groups.

DISCUSSION

In this study our aim was to elucidate the effects that mechanical vibration might have on orthodontic tooth movement. The reason for investigating this topic is that in the literature there has been a great disparity in the reported findings regarding the effects of vibration. Both on a macroscopic and microscopic level, confounding results have been seen. One of the reasons for this might be due to the vast differences in research protocols tested, frequencies utilized, differing or even un-reported force levels applied, and of course the obvious differences seen between the various animal models used.

The reason for investigating the effects of mechanical vibration on orthodontic tooth movement clearly stems from the very foundation that supports the dentition, which is the surrounding alveolar bone.

As far back 1885, Wilhelm Roux spoke of the dynamic ability of bone to adapt to the forces that act upon it, even though to most people this novel concept is unfortunately remembered as Wolff's Law, even if he was not the originator of this idea [37]. Being that the dentition is incased in bone, it makes very logical sense that any effects on the alveolar bone might have a direct effect on the speed at which teeth are able to move through this bone. From this point onwards, however, is where the disparity in the literature begins.

Most people are familiar with the phenomenon associated with loss of bone mass in astronauts who are exposed to prolonged periods of zero gravity. The concept behind this medical condition is the lack of mechanical loading of the bones due to the lack of gravity present, and thus the bone mass decreases. In accordance with this, the very opposite phenomenon is seen with gymnasts who exhibit a much greater loading of bones, and thus have been found to have greater bone density in their long bones.

When Umemura et al. looked at jumping frogs in 1997, he found that increasing the amount of jumps per day did not have any effect on how much bone was produced as long as the frog jumped 5 times in a day when compared to more than one hundred times [38]. As a result he concluded that duration of loading was not a factor. Lanyon in 1984 took it one step further and investigated birds and postulated that bones are able to respond to mechanical stimuli and thus become desensitized easily [39]. With this concept in mind, Rubin et al. in 2002, and again in 2004 investigated the effects of low-magnitude high frequency vibration on trabecular bone formation and found that a significant increase in density was seen [40] [18].

From these aforementioned studies, as well as many others, it is now very widely accepted that vibration has an anabolic effect on bone [18]. With this being said, it seems almost logical that if bone density were to increase as a result of mechanical vibration, then orthodontic tooth movement would in fact decrease as a result, however, opposite findings have been found by various researchers, namely Darendeliler in 1995 [12] and 2007 [14], as well as one of the more recent findings by Nishimura et al. in 2008 [15]. One of the most logical ways to elucidate the reason for these findings would be to evaluate what is going on at the cellular level and see if there is an up-regulation or down-regulation of osteoclasts which are the most important cells involved in bone turnover. However, exploration into this area has produced even more confounding results.

When looking at the effects on osteoclasts, studies have shown that vibration can cause an increase [15], a decrease [41], or even no effect at all on osteoclast numbers [42]. Thus, in our study the aim was to not only determine if mechanical vibration has an effect on orthodontic tooth movement, but if it does, at what frequency does it have the greatest effect. Following these results, the idea was to then look at the bone at a microscopic level to try and understand the biology behind the results.

In this study 5Hz, 10Hz and 20Hz were all evaluated with the same vertical force of 5g applied to the occlusal surface of the maxillary right first molars. These various frequencies were applied to mice in both the experimental groups (concurrent orthodontic force of 10g) and the control groups (no orthodontic force) in order to see if there was any difference in the effects not only on the amount of tooth movement, but also on the actual bone itself.

While there was no difference in the amount of tooth movement between the various experimental groups, there was a trend that was seen: Spring + 5Hz > Spring + 10Hz > Spring + 20Hz > Spring only, which seems to follow the concept that vibration has an anabolic effect on bone, in the fact that as the frequency increases there is a concomitant decrease in the amount of tooth movement seen. However, all of the vibration groups did show more tooth movement than an orthodontic force on its own. From these non-statistical findings, one could infer that the application of vibration at a very low frequency might have a positive correlation with more tooth movement, however as the frequency increases the amount of tooth movement gradually approaches that of no vibration application at all.

While this trend seems to correlate with some prior studies, it also contradicts many others. This research project has not been completed as of yet, and currently the experimental groups are growing in size. The hope is that, as the sample size increases some statistical significance can be found and more conclusive findings can be made.

With regard to Bone Volume Fraction (BV/TV) all of the experimental groups did show statistically significantly lower values when compared to control groups, but this correlates with the fact that due to the orthodontic tooth movement occurring, localized modeling and remodeling is taking place which would account for these findings. However, when trying to determine if one frequency has a preferential effect over another, we were unable to do so because no statistical significance could be found when comparing one experimental group with another. With the addition of more experimental samples being currently tested, this again could change in the near future as sample sizes increase.

What is interesting, however, and almost seems to go against the prior trend that we have seen, is that a statistically significant drop in the bone volume was seen in the 20Hz control group when compared with the untouched baseline group which would appear to lean towards a more catabolic effect as the frequency of vibration increases, however, this finding was not corroborated when looking at tissue density which was not statistically different.

Unfortunately, with the current data available, we are still not able to determine if the application of mechanical vibration will have any clinically relevant effects and if so in which direction they will be. Following the completion of more experimental samples, some new light may be shed on this topic along with the results of the histological staining which is currently underway.

CONCLUSIONS

With the current sample size, we were unable to find any statistical significance between the various experimental groups, and thus the null hypothesis was accepted. The preliminary finding is that no difference in the amount of orthodontic tooth movement is seen when mechanical vibration is applied, compared with that of orthodontic force on its own. If these findings remain the same in the future as more samples are completed, then our findings are similar to those of the recent prospective clinical trial that evaluated the effects of the Tooth Masseuse, but in the near future we will know if this is truly the case.

FIGURES

Figure 1. Application of Orthodontic Force: Ni-Ti spring appliance in the mouth consisting of a Ni-Ti coil spring attached to the maxillary right first molar (left) and both central incisors (right) via two separate segments of 0.004 mm stainless-steel (SS) ligature wire. To prevent any dislodging, the wire around the incisors is secured using a composite resin. Mouth is being held open with a custom mouth-prop fabricated from 0.036” Stainless Steel wire utilized during spring placement. Lips are being retracted with a custom mouth-prop fabricated from 0.017” x 0.025” TMA wire utilized during application of vibration (see below).

Figure 2. Bose Electromechanical Actuator: a feedback-loop controlled, electromechanical actuator (Model 3230, Bose/EnduraTec, Minnetonka, MN) utilized in order to apply unilateral mechanical vibration to the occlusal surface of the maxillary right first molar along the long axis of the tooth.

Figure 3. Application of Mechanical Vibration: tip of electromechanical actuator (Model 3230, Bose/EnduraTec, Minnetonka, MN) is touching the occlusal surface of the maxillary right first molar. Mouth is being held open with a custom mouth-prop fabricated from 0.017" x 0.025" TMA wire utilized during application of vibration.

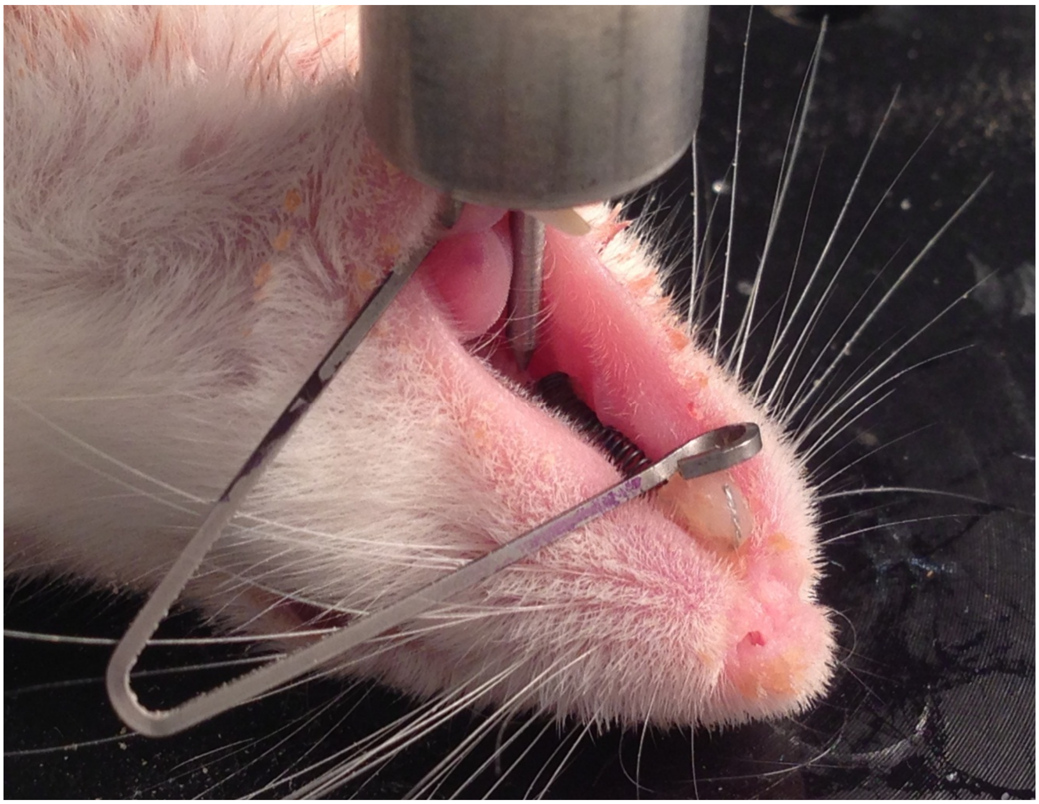


Figure 4. μ-CT image: sample image from the Base Line control group that received no orthodontic force and no application of vibration.

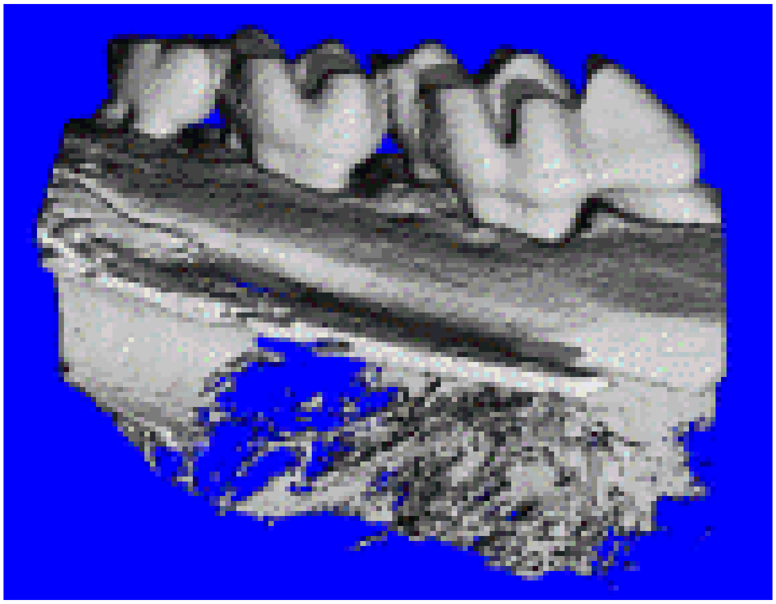


Figure 5. μ-CT image: sample image from the 5Hz Vibration Only control group (no orthodontic force). This image shows that the application of vibration without an orthodontic force will not result in any tooth movement.

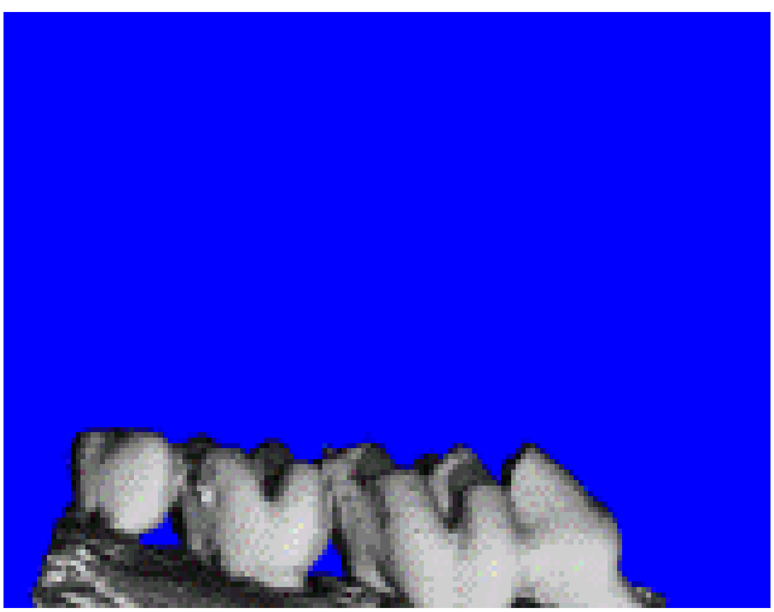
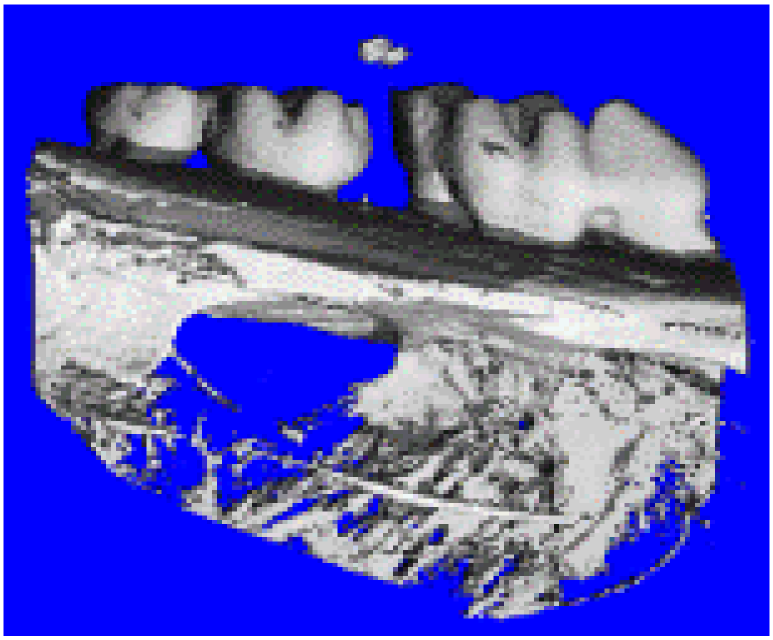


Figure 6. μ-CT image: sample image from the Orthodontic Force & 5Hz Vibration Experimental group. This image shows that the application of vibration along with an orthodontic force results in tooth movement.



TABLES

Table 1. Experimental Group – Spring + 5Hz Vibration: μ-CT data showing the amount of tooth movement and properties of surrounding bone.

Sample Name	BVF (BV/TV)	Tissue Density	1st Molar Movement	Bone Volume
Spring-5Hz - 1	60.8%	1090	0.228	0.096
Spring-5Hz - 2	66.2%	1134	0.200	0.085
Spring-5Hz - 3	55.7%	1155	0.240	0.070
Spring-5Hz - 4	70.2%	1159	0.444	0.072
Spring-5Hz - 5	67.3%	1125	0.206	0.119
Spring-5Hz - 6	66.5%	1121	0.236	0.108
Average	**64.4%**	**1130.718**	**0.259**	**0.091**
SD	5.3%	25.159	0.092	0.020

Table 2. Experimental Group – Spring + 10Hz Vibration: μ-CT data showing the amount of tooth movement and properties of surrounding bone.

Sample Name	BVF (BV/TV)	Tissue Density	1st Molar Movement	Bone Volume
Spring-10Hz - 1	60.8%	1090	0.232	0.096
Spring-10Hz - 2	66.2%	1134	0.423	0.085
Spring-10Hz - 3	65.7%	1155	0.122	0.070
Spring-10Hz - 4	70.2%	1059	0.244	0.072
Spring-10Hz - 5	68.5%	1071	0.266	0.078
Average	**66.3%**	**1101.864**	**0.257**	**0.080**
SD	3.6%	41.348	0.108	0.010

Table 3. Experimental Group – Spring + 20Hz Vibration: μ-CT data showing the amount of tooth movement and properties of surrounding bone.

Sample Name	BVF (BV/TV)	Tissue Density	1st Molar Movement	Bone Volume
Spring-20Hz - 1	57.6%	1122	0.130	0.061
Spring-20Hz - 2	49.3%	1131	0.120	0.048
Spring-20Hz - 3	70.9%	1136	0.370	0.121
Spring-20Hz - 4	62.6%	1112	0.170	0.091
Spring-20Hz - 5	51.3%	1129	0.331	0.081
Spring-20Hz - 6	39.7%	1074	0.151	0.060
Spring-20Hz - 7	57.0%	1122	0.166	0.075
Average	**55.5%**	**1118.188**	**0.205**	**0.077**
SD	10.0%	21.089	0.101	0.024

Table 4. Control Group – Spring Only (no vibration)**:** μ-CT data showing the amount of tooth movement and properties of surrounding bone.

Sample Name	BVF (BV/TV)	Tissue Density	1st Molar Movement	Bone Volume
Spring-NoVib - 1	57.6%	1122	0.330	0.061
Spring-NoVib - 2	49.3%	1131	0.170	0.048
Spring-NoVib - 3	70.9%	1136	0.192	0.121
Spring-NoVib - 4	62.6%	1112	0.144	0.091
Spring-NoVib - 5	51.3%	1129	0.105	0.081
Spring-NoVib - 6	39.7%	1074	0.201	0.060
Spring-NoVib - 7	57.0%	1122	0.193	0.075
Spring-NoVib - 8	47.7%	1051	0.336	0.036
Average	**54.5%**	**1109.822**	**0.209**	**0.072**
SD	9.7%	30.678	0.083	0.027

Table 5. Control Group – No Spring + No Vibration (Base Line)**:** μ-CT data showing the properties of surrounding bone.

Sample Name	BVF (BV/TV)	Tissue Density	Bone Volume
NoSpring-NoVib - 1	84.8%	1174	0.097
NoSpring-NoVib - 2	82.5%	1136	0.109
NoSpring-NoVib - 3	75.9%	1138	0.102
NoSpring-NoVib - 4	77.8%	1130	0.100
Average	**80.3%**	**1144.515**	**0.102**
SD	4.1%	19.685	0.005

Table 6. Control Group – No Spring + 5Hz Vibration (no orthodontic force)**:** μ-CT data showing the properties of surrounding bone.

Sample Name	BVF (BV/TV)	Tissue Density	Bone Volume
NoSpring-5Hz - 1	67.4%	1099	0.081
NoSpring-5Hz - 2	84.6%	1161	0.101
NoSpring-5Hz - 3	84.8%	1188	0.087
NoSpring-5Hz - 4	80.2%	1163	0.088
NoSpring-5Hz - 5	78.8%	1144	0.067
Average	**79.2%**	**1150.964**	**0.085**
SD	7.1%	33.168	0.012

Table 7. Control Group – No Spring + 10Hz Vibration (no orthodontic force)**:** μ-CT data showing the properties of surrounding bone.

Sample Name	BVF (BV/TV)	Tissue Density	Bone Volume
NoSpring-10Hz - 1	75.9%	1131	0.076
NoSpring-10Hz - 2	78.4%	1133	0.101
NoSpring-10Hz - 3	78.6%	1154	0.103
NoSpring-10Hz - 4	77.2%	1149	0.075
Average	**77.5%**	**1141.961**	**0.089**
SD	1.3%	11.427	0.015

Table 8. Control Group – No Spring + 20Hz Vibration (no orthodontic force)**:** μ-CT data showing the properties of surrounding bone.

Sample Name	BVF (BV/TV)	Tissue Density	Bone Volume
NoSpring-20Hz - 1	76.2%	1126	0.076
NoSpring-20Hz - 2	72.2%	1146	0.068
NoSpring-20Hz - 3	76.4%	1135	0.075
NoSpring-20Hz - 4	74.0%	1107	0.072
NoSpring-20Hz - 5	79.0%	1148	0.070
Average	**75.6%**	**1132.493**	**0.072**
SD	2.6%	16.611	0.003

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